8.6.2015

MONDAY, 6/8

Present: Tamannae

Did o/n cultures of AH020 and AH021 in 2 ml LB with 1,5 µl chloramphenicol. Also tried to make o/n culture of AH013 with the old plate colony in 2 ml LB with 0,8 µl kanamycin.

NanoDrop results of the gel purification (Table 1) and the ligations (Table 2) which were made in June 3th.

Table1

Sample	DNA (ng/µl)	A260/A280
AH011	4,8	1,82
AH014 10	3,7	1,55
AH014 20	3,5	1,81
AH015 10	3,6	1,8
AH015 20	3,0	1,95

Table2

Sample	DNA (ng/µl)	A260/A280
AH011	146,1	2,06
AH014 10	751,8	1,89
AH014 20	170,6	1,93
AH015 10	204,1	1,73
AH015 20	224,3	1,71

9.6.2015

TUESDAY, 6/9

Petra & Tamannae
Miniprepped yesterday's o/n cultures AH013, AH020 and AH201. Followed the kit protocol (GeneJET Plasmid Miniprep Kit)
Nanodrop results for the miniprepped plasmids:

Table1

Plasmid	Concentration (ug/ul)	A260/A280
AH013	87,7	1,87
AH020	287,6	1,78
AH021	134,9	1,85

Made a new plate culture of the leftovers of AH013 o/n culture. However, team member didn't trust that the AH013 would be the correctly ligated so they decided to produce another one.

Started new restriction digestions to produce AH011, AH013, AH014 and AH015. Used Linearized plasmid backbone pSB1K3 as AH008. Followed the protocol by adding DNA and water according to the table:

Table5

Plasmid	Original concentration (ng/ul)	Added DNA (ul), -> 250 ng	Addedwater (ul)
AH001	51,4	4,9	16,6
AH002	46,9	5,4	16,1
AH003	58,3	4,3	17,1
AH004	43,2	5,8	15,7
AH005	44,8	5,6	15,9
AH006	55,0	4,6	16,9
AH008	25,0	10,0	11,5

Ligated restricted plasmids:
AH001+AH006+AH008=AH011
AH002+AH003+AH008=AH013
AH002+AH004+AH008=AH014
AH002+AH005+AH008=AH015

Ligation mixes:
2,5 ul each DNA (7,5 ul DNA in total per mix)
1ul buffer
0,5 ul ligase
1 ul water

Followed the ligation protocol.

Stored ready ligations in the freezer.

10.6

WEDNESDAY, 6/10

Petra

Checked yesterday's AH013 culture. Growing, looks good. Transferred to the fridge.

Transformed yesterday's ligations AH011, AH013, AH014 and AH015. Followed the protocol. No control was made.
Plated the transformants to kanamycin plates and stored to incubator overnight.

11.6.2015

THURSDAY, 6/11

Present: Tamannae
Transformations were successful (at least there are several colonies in each plate).

Picked 5 colonies from each transformant (AH011, AH013, AH014, AH015) to do o/n cultures in 2 ml LB with Kanamycin (0,8 µl). One colony from AH013 (3) and AH014 (1) were red, so they probably don't contain the right inserts (but I wanted to see that fact in the gel).

Also did 495 ml SOB following the protocol, 10 ml 1 M MgCL2 and 20 ml 1 M glucose which were sent to autoclave. pH were 7.01 after adding 95 µl 2 M NaOH.